Soil Biol. Biochem. Vol. 17, No. 3, pp. 297–302, 1985
Printed in Great Britain.

0038-0717/85 $3.00 + 0.00

SOIL MICROBIAL BIOMASS AND SELECTED SOIL ENZYME ACTIVITIES: EFFECT OF FERTILIZATION AND CROPPING PRACTICES

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(*Accepted* 25 *October* 1984)

Summary—Selected microbiological properties of soils receiving different fertilizer management regimes were studied from adjoining wheat farms in the highly productive Palouse region of eastern Washington. Since 1909, the only N input to the soil of Farm Management System 1 has been through leguminous green manure crops consisting most recently of Austrian winter peas (*Pisum sativum* ssp. *arvense* L., Poir), plus native soil fertility for N and all other plant nutrients. The soil of Farm Management System 2 received regular applications of anhydrous ammonia, P and S at recommended rates for the last 30 yr. There were no differences in numbers of soil microorganisms as determined by plate counts; however, soil from management system 1 had significantly higher levels of urease, phosphatase and dehydrogenase at all three samplings and significantly higher soil microbial biomass at the first two samplings. The data indicate that management system 1 soil had a larger and more active soil microflora.

INTRODUCTION

A major agricultural research priority is to sustain soil productivity and to develop better methods to monitor changes in soil physical, chemical and biological properties as affected by management (Larson *et al.*, 1981). It is well established that crop rotation and organic amendments to the soil can positively affect soil productivity (Smith, 1942; Anonymous, 1957). It is thought that these benefits result from increased fertility, microbial biomass and microbial activity. However, there has been little research to document the effects of green manure crops in the rotation on soil enzymes and soil microorganisms. Also, it is difficult to link these measurements to soil productivity.

Verstraete and Voets (1977) showed yields of winter wheat were positively related to soil phosphatase activity while sugar beet yields were negatively related to soil urease activity. They also found that green manure plus barn-yard manure amendments increased soil urease and phosphatase activities. Martyniuk and Wagner (1978) used dilution plate counts to determine the long-term effect of manuring and crop rotations on soil microorganisms using samples from the Sanborn Field plots which were established in 1888. Soil from the treatments that received manure had higher numbers of bacteria and actinomycetes than those that did not. Soil plate counts from the Morrow plots established in 1876 showed a trend for increased numbers of bacteria when manure was added. More legume crops in rotation raised both the soil organic matter and N content (Anonymous, 1957). High soil organic matter strongly correlates with high levels of soil fertility, productivity and tilth (USDA, 1980). Leguminous green manure crops add N and organic matter to the soil. Microbial degradation and mineralization of organic matter derived from a leguminous green manure crop provides a way for nutrients to become available to future crops.

A 320-ha wheat (*Triticum aestivum*) farm 30 km southwest of Spokane, Washington, was identified in the USDA Report on Organic Farming (1980) as a farm which had been managed without the use of inorganic fertilizers and only limited use of pesticides since the farm was broken out of sod in 1909. The farm is located in a highly productive area where dryland winter wheat yields range from 3000 to 6000 kg ha^{-1} (Papendick and Miller, 1977). The farm management system (management system 1) relied upon green manure crops of Austrian winter pea (*Pisum sativum* ssp. *arvense* L. Poir), crop rotations, and native soil fertility for plant nutrients. No inorganic fertilizer or animal wastes were applied. The crop rotation and fertilizer use on the two farms were determined from averages of all crops on the respective farmers' fields since 1974 (Andrea G. W. Patten, unpublished M.S. thesis, Washington State University, 1982). Management system 1 crop rotation was: winter wheat–spring pea (*Pisum sativum*)–Austrian winter pea. The Austrian winter pea was always used as a green manure crop. Summer fallow use on management system 1 averaged every sixth year, but was replaced by Austrian winter peas when not needed for water conservation. The adjoining 525-ha field (management system 2) received recommended rates of fertilizers and pesticides. Fertilizer applied to winter wheat included N, P and S applied at 96, 34 or 16 kg ha^{-1}, respectively. No fertilizer was applied to spring peas. Management system 2 crop rotation was winter wheat–spring pea. Summer fallow was used when necessary due to climatic conditions. These two management systems

differed in managers, fertilizer use and crop rotations, while they employed similar tillage operations and crop varieties.

The different farm management systems could have a variety of effects on soil microorganisms and soil microbial activity. Our objective was to determine if the two management systems affected the numbers and activities of soil microorganisms as measured by dilution plate counts, enzyme assays and microbial biomass.

MATERIALS AND METHODS

Soil from adjacent fields of the two farm management systems was sampled three times during the year (21 July 1981, under maturing winter wheat; 11 November 1981, after plowdown of winter wheat straw and stubble; and 11 May 1982, under a crop of spring peas). The three sampling dates were chosen to determine if any differences in biological activity between the soils were consistent and to provide extremes, if any, in measurements of biological activity. In the Palouse region, the soil is normally dry in July, wet and cooling in November, and moist and warming in May. The soil from both fields is a Naff (fine-silty, mixed, mesic Ultic Argixeroll). Due to a slope of approximately 10% on the field sites sampled, a split block with time experimental design was used (Little and Hills, 1978) and plots were placed mid-slope across the adjacent fields. The 6×15 m plots were located 15 m away from the boundary line between the farms and replicated eight times. Twenty soil cores, 15×2 cm dia, were pooled from each plot at sampling. The 0–15-cm sampling depth was chosen because the surface horizons of a profile tend to have the highest levels of biological indicators and this depth generally corresponded to the tillage depth for both farms. The samples were stored on ice in transit, sieved (<2 mm) and stored at 4°C for no longer than 1 week before assay of enzymes and organisms. All biological estimations were conducted on fresh soil samples so that comparisons between measurements could be made. Also, the literature presents conflicting evidence on the effects of air drying on soil enzyme activity.

The serial dilution technique was used for all soil plate counts. Soil microorganisms were estimated with soil extract agar for total aerobes (Allen, 1959), Martin's medium for fungi (Martin, 1950) and the medium of Kuster and Williams (1964) supplemented with the antibiotics suggested by Williams and Davies (1965) for actinomycetes. Denitrifiers were assayed with the most probable number (MPN) technique (Alexander, 1965). The MPN technique was also used to measure *Nitrosomonas* sp. (Alexander and Clark, 1965). A slight modification in color reagent was used for *Nitrosomonas* sp. determination. Technicon® color reagent (sulfanilamide, phosphoric acid and *N*-1-naphthylethylenediamine dihydrochloride) was used to detect NO_2^--N rather than the Griess–Ilosvay Reagent (Technicon® Industrial Systems, 1973).

Urease activity was measured by Klein and Koths' (1980) non-buffer procedure. Acid phosphatase activity was measured with the method of Tabatabai and Bremner (1969) except that fresh instead of dried soil was used. Dehydrogenase activity was estimated with the procedure of Casida *et al.* (1964). All enzyme determinations were replicated three times.

A modification of Jenkinson and Powlon's (1976b) chloroform fumigation procedure was one method used to determine soil microbial biomass. Only 90 g of soil was used rather than the 250 g specified in the original procedure. After the fumigation or control treatment, the samples were brought to a water content equivalent to -0.03 MPa. Soils were held at 24°C for the standard 0–10- and 10–20-day periods. Microbial biomass was calculated by subtracting CO_2 evolved during the 0–10- or 10–20-day incubation period of the control soil from CO_2 evolved during the 0–10-day incubation of the fumigated soil. A k value of 0.41 was used for conversion of CO_2-C to biomass C (Anderson and Domsch, 1978).

A modification of Ross *et al.* (1980b) was used for the determination of N mineral flush (N flush). Nitrogen flush is an indication of microbial biomass. Nitrogen flush is the difference in mineral N released between fumigated and non-fumigated control soils incubated for the same length of time. After CO_2 determinations on biomass soils at day 20, duplicate samples of soil were extracted with 2 M KCl to measure NH_4-N (Connetta *et al.*, 1976) and NO_3^--N (APHA, 1975).

Air-dried soil samples were extracted with 2 M KCl and NH_4^+-N (Connetta *et al.*, 1976) and NO_3^--N (APHA, 1975) determined in the extracts. Kjeldahl N was measured using the procedure of Conetta *et al.* (1976). The Modified Mebius Procedure of Nelson and Sommers (1982) was used for organic C determination. Soil pH was measured with a glass electrode on a one-to-one (w/w) soil to deionized water ratio.

All results are reported on a g^{-1} oven (110°C) dry weight of soil basis. A protected least significant difference was used to analyze all pairwise comparisons (Little and Hills, 1978).

RESULTS

Soil pH, organic C and Kjeldahl N of management system 1 was significantly higher than management system 2 while mineral N was significantly lower (Table 1). Both management systems yielded close to long-term averages for the general area (Table 2). Management system 1 did not use chemical fertilizers, yet yields were above the average for farms in the general area. However, management system 1 was not cropped to wheat as often as the average because Austrian winter pea was used in the rotation.

Total counts of viable soil microorganisms, fungi or actinomycetes showed very minor differences be-

Table 1. Soil chemical properties of adjacent management systems

Value	Management system 1	Management system 2
pH	5.85*	5.71
Mineral N ($\mu g\ g^{-1}$)	11.46	28.50**
Percent organic C	1.14**	0.91
Total Kjeldahl N ($\mu g\ g^{-1}$)	1179.00*	1066.00

*,**Significant at the 0.05 and 0.01 levels, respectively. Means are averages of three sampling dates. The pH mean was determined from the average of the H^+ activity.

Table 2. Average estimates of recent and long-term yields on management system 1, 2, and general area farms[a]

Crop	Management system 1		Management system 2		General area	
	1974–81	1950–81	1976–81	1950–81	1970–81	1950–81
	(Mg ha^{-1})[b]		(Mg ha^{-1})[b]		(Mg ha^{-1})[b]	
Winter wheat	4.76	4.76	4.22	3.81	4.42	3.40
Spring peas	2.14	2.04	2.09	1.81	2.00	1.70

[a]We used Farm Manager estimates for recent and long-term yields on management system 1 and 2. The general area estimates were derived from farm and agricultural organization records. (Andrea G. W. Patten, unpublished M.S. thesis. Washington State University, 1982).
[b]Mg = metric tonne.

tween the two management systems throughout the year (Table 3). In November, management system 1 soil had significantly more fungi, but the difference between the fungal counts for the two soils was not great. Fungal numbers were about the same on the three sampling dates, while total microbial and actinomycete counts appeared to increase from July to May.

Nitrosomonas spp numbers tended to be higher in management system 2 soil on the three sampling dates, but reached significance only in May (Table 3). Numbers of *Nitrosomonas* spp stayed the same in management system 1 soil on the three sampling dates, while management system 2 soil showed an increase in May as compared to the July and November samplings. Potential denitrifier numbers were the same in both soils and remained the same on the three sampling dates.

Management system 1 soil had significantly higher levels of urease, phosphatase and dehydrogenase activity than management system 2 soil on the three sampling dates (Table 3). Urease activity remained stable in both soils but phosphatase activities in both soils decreased from July to November and was at the same level in May. Dehydrogenase activities in both soils stayed at the same level for July and November and had decreased in May.

Management system 1 soil had significantly higher levels of microbial biomass in the July and November samples when the CO_2 evolution data from the 10- to 20-day control soil was used (Table 3). In May, management system 1 soil tended to have higher microbial biomass, but the difference was not significant. Biomass levels decreased in both soils from the July to May sampling dates. The N flush results were similar to those obtained for microbial biomass. Management system 1 soil had significantly higher N flush levels at all three sampling dates (Table 3), and N flush levels also decreased in both soils over the sampling period.

DISCUSSION

It was not surprising that soil dilution plate counts showed virtually no difference between the management practices. Verstraete and Voets (1977) reported similar results. They studied the effects of crop rotations and fertilizer treatments on soil microbial numbers for 7 yr using soil dilution plate counts and found no differences. They attributed their results to the inherent errors present in the soil dilution plate count technique which masked differences among treatments.

In July and November the amounts of mineral N and members of *Nitrosomonas* spp were similar in the two soils. That *Nitrosomonas* sp. were significantly higher in May in management system 2 soil likely resulted from the higher concentration of NH_4^+-N

Table 3. Plate counts, most probable number, enzyme and biomass assays for soils in adjacent management system on three sampling dates

	July 1981 System		November 1981 System		May 1982 System	
	1	2	1	2	1	2
Plate counts						
Total (10^7)	2.57	2.12	5.20	5.73	5.70	6.13
Fungi (10^5)	3.70	2.79	4.07**	2.69	2.97	2.84
Actinomycetes (10^6)	4.02	3.30	ND	ND	7.39	7.21
Most probable number						
Nitrosomonas (10^4)	2.32	15.50	3.56	8.05	2.41	31.60**
Denitrifiers (10^5)	3.41	2.25	0.98	1.28	1.31	2.29
Enzyme and biomass assays						
Urease[a]	2.51**	1.70	2.76**	1.71	3.01**	1.57
Phosphatase[b]	3.47**	3.04	2.24**	1.76	2.06**	1.53
Dehydrogenase[c]	8.56**	5.90	9.58**	5.73	7.25**	4.27
Biomass 1[d]	191.00*	109.00	114.00	38.00	51.00	45.00
Biomass 2[e]	314.00**	248.00	237.00**	136.00	142.00	124.00
N flush (μg N g^{-1})	14.50**	7.16	12.00**	5.32	11.20**	2.46

[a]10^{-2} μmol NH_4^+-N g^{-1} min^{-1}; [b]10^{-2} μmol *p*-nitrophenol g^{-1} min^{-1}; [c]10^{-5} μmol triphenylformazan g^{-1} min^{-1}; [d]μg C g^{-1} using 0–10-day control; [e]μg C g^{-1} using 10–20-day control; ND = not determined.
*,**Significant at the 0.05 and 0.01 levels, respectively, within a given sampling date. Significance between sampling dates are not indicated but are discussed in the text.

substrate present. In May, management system 2 soil had three times the mineral N level as the management system 1 (data not shown) due to anhydrous ammonia fertilization of spring grain (only the plot site was planted to peas on management system 2).

Soil enzymes originate from animal, plant and microbial sources. There is a widespread implied assumption that soil enzymes are derived primarily from soil microorganisms (Ladd, 1978). If this is true, then the assay of several soil enzymes should be an indicator of general microbial populations and activities.

Although urease activity was higher in the soil from management system 1, soil urease activity varied little among sampling dates in this study. McGarity and Myers (1967) obtained similar results when they compared urease activity in soil samples taken from five great soil groups from New South Wales in winter and spring and found that urease activity in four of them did not vary between the two sampling dates. In Australia, these sampling dates would represent two periods of contrasting biological activity. The values of urease activity found here (Table 3) agree with selected values reported in the literature (Table 4).

Both values found in this study and literature values are reported as μmol product formed $g^{-1}\,min^{-1}$. It is suggested that researchers adopt these units (or μmol substrate utilized $g^{-1}\,min^{-1}$) for reporting soil enzyme activities. These units closely agree with those suggested by the Commission on Enzymes of the International Union of Biochemistry (μmol product formed or substrate utilized min^{-1}, Dobrogosy, 1981) except the denominator also includes gram dry weight of soil.

Phosphatase activity fluctuated over time (Table 3) in both soils. There was a peak of activity in July, a significant decrease in activity in November, and an apparent further decrease in May, although the latter decrease was not significant. Harrison and Pearce (1979) found a similar summer peak of activity and suggested that the increases were due mainly to phosphatase production by roots and soil microorganisms. The phosphatase values reported here (Table 3) also agree with selected literature values (Table 4).

Dehydrogenase enzymes appear to be linked with microbial activity associated with initial breakdown of organic matter (Ross, 1971). Ross (1970) stated that dehydrogenase activity appeared to be more dependent on the metabolic state of the soil or on the biological activity of the microbial population than on any free enzyme present. Dehydrogenases in soil are believed to be intracellular enzymes mainly linked with microbial respiratory processes. The dehydrogenase assay also has a potential advantage because no additional substrate is added to the soil. Therefore, there is no preferential stimulation of any particular group of soil microorganisms (Stevenson, 1959). For these reasons, dehydrogenase is consid-

Table 4. Selected values from the literature for urease, phosphatase, and dehydrogenase activity, biomass, and N flush

Measurement	Value[a]	Source
Urease	1.57–3.01	This study
(10^{-2} μmol NH_4^+-N $g^{-1}\,min^{-1}$)	1.58	Balasubramanian *et al.* (1972)
	3.88–7.46	Douglas and Bremner (1971)
	0–6.18	Zantua and Bremner (1975)
	2.09–3.78	Klein and Koths (1980)
	1.50–7.86	Speir (1977)
Phosphatase	1.53–3.47	This study
(10^{-2} μmol *p*-nitrophenol $g^{-1}\,min^{-1}$)	0.64–1.51	Eivazi and Tabatabai (1977)
	0.93–1.33	Nielsen and Eiland (1980)
	2.03–2.53	Klein and Koths (1980)
	2.56–4.68	Neal (1973)
	2.83–9.92	Tabatabai and Bremner (1969)
Dehydrogenase	4.27–9.58	This study
(10^{-5} μmol triphenylformazan $g^{-1}\,min^{-1}$)	1.98–13.71	Sparling (1981)
	2.04–4.03	Khan (1970)
	3.54–31.91	Stevenson (1959)
	14.26–60.88	Casida *et al.* (1964)
	30.00–1530	Ross (1973)
	79.00–283	Ross (1970)
Biomass	124–314	This study
(μg C g^{-1})	30–750	Lynch and Panting (1980a)
	43–1130	Jenkinson and Powlson (1976a)
	48–778	Oades and Jenkinson (1979)
	60–480	Ayanaba *et al.* (1976)
	88–398	Sparling (1981)
	170–1180	Jenkinson and Powlson (1976b)
	272–315	Jenkinson and Powlson (1980)
	430–1400	Lynch and Panting (1980a)
	480–770	Lynch and Panting (1980b)
	530–2780	Ross *et al.* (1980b)
	950–1621	Ross *et al.* (1980a)
N flush	2.46–14.5	This study
(μg N g^{-1})	22–153	Jenkinson *et al.* (1979)
	74–167	Ross *et al.* (1980a)
	59–167	Ross *et al.* (1980b)

[a]All literature values were converted to similar units.

ered to be an index of endogenous soil microbial activity (Moore and Russell, 1972).

The dehydrogenase activity in both soils stayed the same from July to November, but there was a significant decrease in May indicating the soil microbes were at a lower activity in May. Dehydrogenase values reported here (Table 3) agree with selected values from the literature (Table 4).

All three enzyme activities measured were significantly higher in the soil from management system 1 than management system 2. These results indicate that microbial activity was higher in management system 1.

For the chloroform fumigation, Jenkinson and Powlson (1976b) suggested the use of the 10–20-day control due to the large initial respiration rates in the 0–10-day control likely caused by sampling and handling procedures. Ayanaba *et al.* (1976) also used the 10–20-day control. In this study, more CO_2 was evolved from some of the control soils than from the fumigated soils during the 0–10-day period (data not shown). For these reasons, only the 10–20-day control soil respiration data are discussed here.

There was no apparent flush of microbial growth in May as compared to November as seen from the biomass data (Table 3), presumably because of the cold and wet spring climate. May samples were at −0.03 MPa water potential when brought into the laboratory and, thus, at a high water content. In the month preceding the May sampling, the departure from average temperature was −1.4°C and the departure from normal precipitation was +28.2 mm (U.S. Environmental Data and Information Service, 1981). Nitrogen flush, which also represents a measure of biomass, did not show an increase in May, either, which tends to support the biomass data. Finally, the enzyme dehydrogenase, which is an indicator of biological activity (Moore and Russell, 1972), had decreased in both soils in May, suggesting that not only was the biomass at a lower level, but that the biomass present was less active. Ross *et al.* (1981) found similar depressed biomass levels in the spring and higher levels in the summer–autumn.

The use of sieved soil for microbial biomass should not affect the absolute difference between samples. Selected literature values of biomass C (Table 4) tended to be higher than those reported here (Table 3). When biomass C is presented as a percentage of organic C in soil, the values are usually 1–4% with an average of 2–3% of the soil organic C being biomass C (Jenkinson and Ladd, 1981). The biomass levels obtained in this study ranged from 1.1 to 3.5% of the soil organic C (data not shown). This agreed well with the data of Jenkinson and Ladd (1981). The lower levels of soil microbial biomass obtained in these soils might be due to lower levels of organic C present in the soil.

Only a few measurements of mineral N flush produced by fumigation have been reported in the literature. The literature values of N flush (Table 4) were higher than values reported here. This may be due to the longer incubation time of 20 days used in this study instead of the customary 10. Immobilization of N could have occurred especially in the fumigated sample, which would decrease the N flush measured. Also, if N flush is a measure of microbial biomass, then a small biomass would have a low N flush. Because these soils had a lower biomass than the selected literature values, one would expect a lower value for the N flush.

The biomass and N flush data would indicate that management system 1 soil has a larger amount of soil microorganisms present. The soil enzyme activities measured in this study were always higher in management system 1. If these enzyme activities can be implied as an indication of soil microbial activity (especially dehydrogenase), then management system 1 soil seems to have a more active soil microflora. This should be expected since management system 1 soil had slightly higher organic C levels and both soil microorganisms and plants must rely more heavily on microbial mineralization to supply nutrients. However, differences between the two soils, although usually significant, were generally small.

Acknowledgements—In cooperation with the College of Agriculture Research Center, Washington State University, Scientific Paper No. 6773.

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